

Lung cancer with concurrent *EGFR* mutation and *ROS1* rearrangement: a case report and review of the literature

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Abstract: *ROS1* rearrangement has recently emerged as a new molecular subtype in non-small cell lung cancer, and is predominantly found in lung adenocarcinomas compared with other oncogenes such as *EGFR*, *KRAS*, or *ALK*. Patients who have both mutations are extremely rare. Here we report a 50-year-old female diagnosed with adenocarcinoma with sarcomatoid differentiation, who was shown to have *EGFR* and *ROS1* mutations. The patient was treated surgically and received three cycles of adjuvant postoperative chemotherapy. In addition, we reviewed the previously reported cases and related literature. This presentation will provide further understanding of the underlying molecular biology and optimal treatment for non-small cell lung cancer patients with more than one driver mutation.

Keywords: non-small cell lung cancer, *EGFR* gene mutation, *ROS1* fusion gene

Introduction

Non-small cell lung cancer (NSCLC) is still the leading cause of cancer-related deaths worldwide. The prognosis is poor for most patients with NSCLC, even with the most current treatment regimens, which include surgery, chemotherapy, and radiation. Targeted molecular therapy is effective for advanced NSCLC patients with associated gene mutations. Although driver genes, including epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene (*KRAS*), are common molecules in lung adenocarcinomas, the c-ros oncogene 1 receptor tyrosine kinase (*ROS1*) rearrangement has been identified in only 1%–2% of NSCLC cases.^{1,2} Previous studies have suggested that *ROS1* fusion is exclusive to *EGFR*, *KRAS*, or *ALK* mutations and presents in a greater percentage of tumors that lack other genetic changes associated with lung cancer.^{3–5} Nevertheless, at least four patients with an *EGFR* mutation and *ROS1* fusion have been reported thus far in the world literature.⁶ The patient reported herein is the fifth case, and also the first case with an *EGFR* exon 21 L858R point mutation and *CD74-ROS1* fusion gene. Little is known about the prognostic value, clinical presentation, predictive value for different therapy regimens, and the genetic heterogeneity for two gene-positive NSCLC patients. All protocols in the present study were approved by the Human Clinical and Research Ethics Committees of the Zhejiang Corps Hospital (Jiaying, People's Republic of China). The patient provided written informed consent.

Case report

A 50-year-old female who had never smoked was evaluated for persistent cough and shown by computed tomography (CT) scanning to have a 32 mm tumor in the right lower

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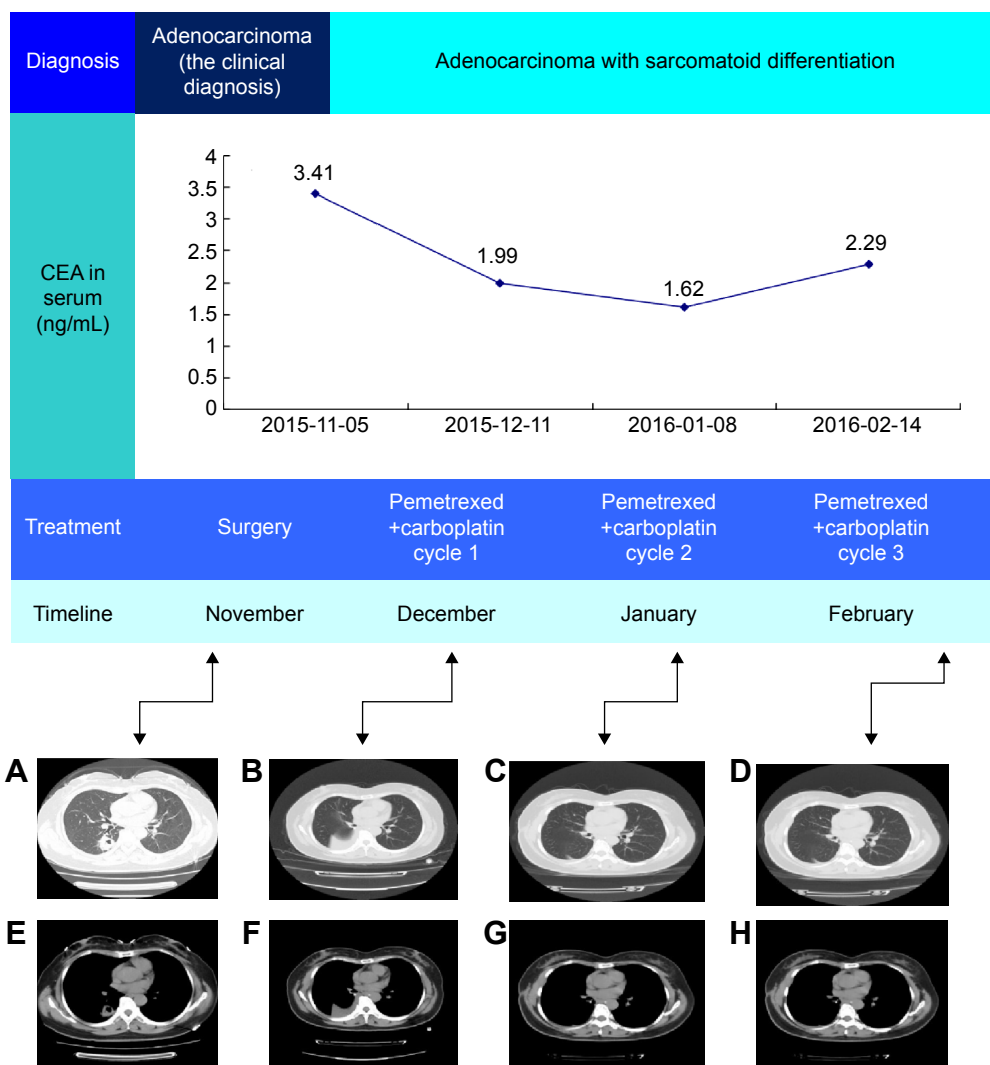


Figure 1 Treatment of lung adenocarcinoma with sarcomatoid differentiation using different chemotherapy regimens and results of monitoring the CEA levels.

Notes: (A–D) Lung CT scans from (A) November 2015, (B) December 2015, (C) January 2016, and (D) February 2016. (E–H) CT scans of the mediastinum from (E) November 2015, (F) December 2015, (G) January 2016, and (H) February 2016.

Abbreviations: CEA, carcinoembryonic antigen; CT, computed tomography.

lobe of the lung in November 2015 (Figure 1). No significant medical history was reported and no abnormalities were found on physical examination. Imaging examinations, including abdominal CT, brain magnetic resonance imaging, and bone emission computed tomography, were normal and blood laboratory testing was within normal limits, including a biochemistry and coagulation profile, and routine hematologic parameters.

The patient underwent resection of the right lower lobe and en bloc resection of the associated hilar and mediastinal lymph nodes by video-assisted thoracic surgery. The postoperative course was uneventful and the patient recovered quickly. The postoperative pathology showed that the tumor was an adenocarcinoma with sarcomatoid differentiation (Figure 2). Immunohistochemistry staining was positive for the following markers: vimentin; thyroid nuclear factor 1; P63;

cytokeratin 7; and cytokeratin 5/6 (Table 1 and Figure 2). The tumor was stage Ib ($T_{2a}N_0M_0$). Gene detection for mutations was performed on a formalin-fixed, paraffin-embedded tumor specimen by next-generation sequencing and fusion genes, and *c-Met* 14 skipping mutation by polymerase chain reaction or fluorescence in situ hybridization on portions of the adenocarcinoma and sarcomatoid differentiation, respectively. A variant of the *ROS1* translocation (Table 2 and Figure 3) and the *EGFR* exon 21 L858R point mutation were detected (Table 3 and Figure 3). The patient received three cycles of postoperative adjuvant chemotherapy. No recurrence of the tumor was noted by CT scanning during 3 months of follow-up care (Figure 1). The CEA level ranged from a pretreatment level of 3.41 ng/mL to a postoperative level of 2.29 ng/mL (Figure 1).

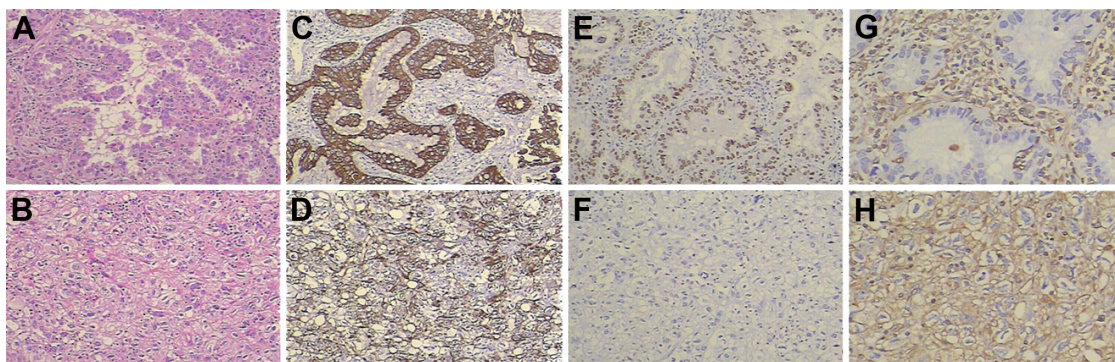


Figure 2 The hematoxylin-eosin staining and the immunohistochemistry in the part of adenocarcinoma and sarcomatoid differentiation.

Notes: (A) The hematoxylin–eosin staining revealed that tumor cells were lung adenocarcinoma ($\times 100$). (B) The hematoxylin–eosin staining revealed that tumor cells were sarcomatoid differentiation ($\times 100$). (C) Immunohistochemical examination revealed that tumor cells were positive for monoclonal anti-CK7 antibody in a portion of the adenocarcinoma ($\times 100$). (D) Immunohistochemical examination revealed that tumor cells were positive for monoclonal anti-CK7 antibody in a portion of the sarcomatoid differentiation ($\times 100$). (E) Immunohistochemical examination revealed that tumor cells were positive for monoclonal anti-TTF-1 antibody in a portion of the adenocarcinoma ($\times 100$). (F) Immunohistochemical examination revealed that tumor cells were positive for monoclonal anti-TTF-1 antibody in a portion of sarcomatoid differentiation ($\times 100$). (G) Immunohistochemical examination revealed that tumor cells were positive for monoclonal antivimentin antibody in a portion of adenocarcinoma ($\times 100$). (H) Immunohistochemical examination revealed that tumor cells were positive for monoclonal antivimentin antibody in a portion of sarcomatoid differentiation ($\times 100$).

Discussion

The *EGFR* gene, which is located on the 12–14 region of short arm of chromosome 7, consists of 28 exons, and most mutations are located within exons 19–21 of the tyrosine kinase domain.^{7–9} Point mutations in exon 21 and overlapping deletions in exon 19 account for ~85% of all mutations.^{10,11}

The *ROS1* rearrangement in NSCLC was discovered by Rikova et al.¹² The fusion partners include CD74-, SLC34A2-, SDC4-, EZR-, FIG-, TPM3-, LRIG3-, and KDELR2-. CD74- is the most common fusion partner in NSCLC.³ *ROS1* (chromosome 6q22) encodes a receptor tyrosine kinase that belongs to the insulin receptor family and stimulates downstream signaling via the mitogen-activated protein kinases pathway, resulting in enhanced cell growth, proliferation, and decreased apoptosis. The frequency of *ROS1* rearrangements ranges from 0.9% to 1.7% in an unselected NSCLC population.^{1,13,14} However, the frequency increases from 3.9% to 7.4% in lung adenocarcinoma patients with wild-type *EGFR/KRAS/ALK*.^{4,5}

The *EGFR* tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, and icotinib, have been widely used as first-line treatment and have higher sensitivity compared to platinum-based chemotherapy in advanced NSCLC patients

with *EGFR* mutations.^{15–17} Deletions in exon 19 and L858R point mutations in exon 21 are the most sensitive mutations with a clear benefit from *EGFR* TKI treatment.

ROS1-positive patients do not benefit from treatment with *EGFR* TKIs.^{4,5} In contrast, patients appear to benefit from treatment with crizotinib, an orally bioavailable anaplastic lymphoma kinase (ALK) inhibitor.^{18,19} Crizotinib is currently undergoing Phase III clinical trials globally. It is anticipated that *ROS1*-positive NSCLC behaves in an analogous manner to *EGFR* mutant NSCLC, and crizotinib could represent an advance in NSCLC treatment.

Patients who have a *ROS1* gene fusion and *EGFR* mutation are extremely rare. Previous studies have suggested

Table 2 Gene mutation identified by next-generation sequencing of the patient

Gene	Type
<i>EGFR</i> mut	L858R
<i>KRAS</i> mut	Wild type
<i>BRAF</i> mut	Wild type
<i>PIK3CA</i> mut	Wild type
<i>NRAS</i> mut	Wild type
<i>KIT</i> mut	Wild type
<i>PDGFRA</i> mut	Wild type
<i>ERBB2</i> mut	Wild type
<i>DDR2</i> mut	Wild type
<i>ALK</i> mut	Wild type
<i>RET</i> mut	Wild type
<i>FLT3</i> mut	Wild type
<i>DNMT3A</i> mut	Wild type
<i>NPM1</i> mut	Wild type
<i>ABL1</i> mut	Wild type
<i>SMO</i> mut	Wild type
<i>TSC1</i> mut	Wild type

Abbreviation: mut, mutation.

Table 1 Primary antibodies used for immunohistochemical staining

Antibody	Clone	Dilution	Purchased from
Vimentin	EP21	1:100	Zymed Laboratories, Inc.
TTF-1	SPT24	1:100	Zymed Laboratories, Inc.
P63	UMAB4	1:100	Zymed Laboratories, Inc.
CK7	EP16	1:100	Zymed Laboratories, Inc.
CK5/6	D5/16B4	1:100	Zymed Laboratories, Inc.

Notes: Zymed Laboratories, Inc. (South San Francisco, CA, USA).

Abbreviations: CK, cytokeratin; TTF-1, thyroid nuclear factor 1.

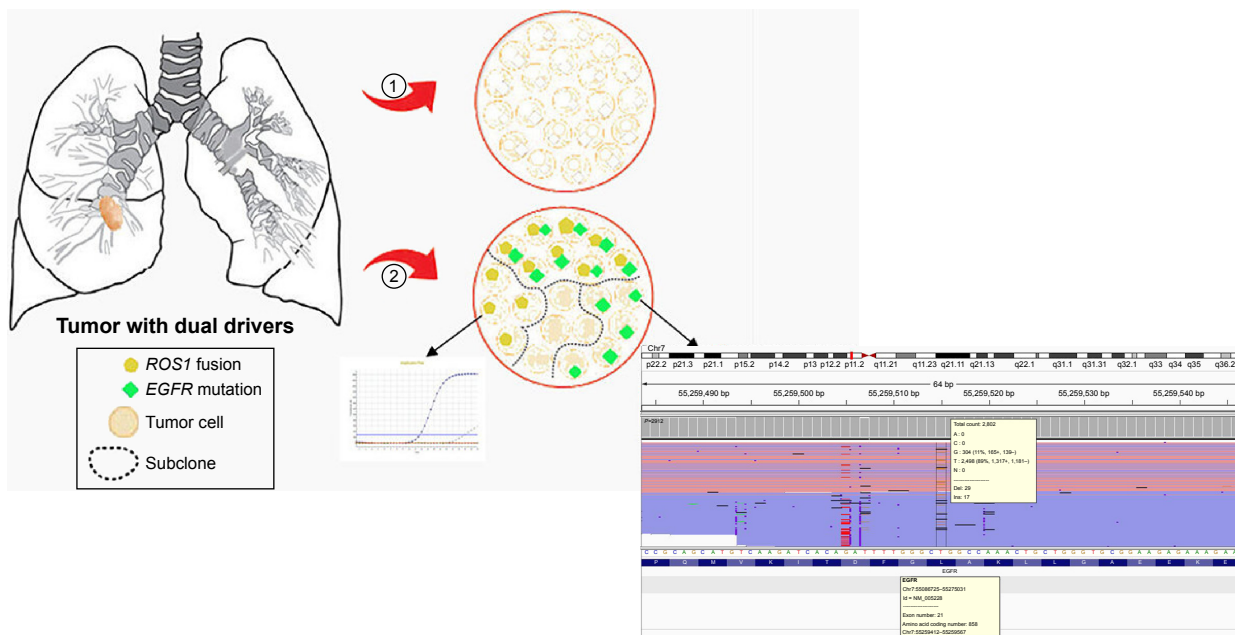


Figure 3 Schema shows tumor with dual drivers (*CD74-ROS1* fusion gene by polymerase chain reaction and *EGFR* exon 21 L858R point mutation by next-generation sequencing) in a portion of the adenocarcinoma (1), and tumor with no driver or unknown driver in a portion of the sarcomatoid differentiation (2).

that an *EGFR* mutation and *ROS1* gene fusion are mutually exclusive molecular events. We reviewed the literature for both *EGFR* and *ROS1* mutations in NSCLC and found only five cases, including the present case,⁶ as shown in Table 4. Indeed, this is the first case in the Han Chinese population identified with a concurrent *EGFR* exon 21 L858R point mutation and a *ROS1* fusion gene with a fusion partner (*CD74*-). Table 4 shows the clinical features of patients with the concomitant genes presented in our study include never-smokers (5/5), adenocarcinoma (4/5), and adenocarcinoma with sarcomatoid differentiation (1/5), exon 19 (3/5), 20 insertion (1/5), and an exon 21 (1/5) mutation of *EGFR*. Two patients with advanced NSCLC harboring concomitant *ROS1* rearrangements and an *EGFR* exon 19 deletion achieved a partial response after first-line gefitinib treatment. The patient presented herein underwent surgery and received three cycles of postoperative adjuvant chemotherapy based on pathology stage Ib and unclear results

Table 3 Noteworthy results identified by PCR or FISH of the patient

Gene	Type
<i>ALK</i> fusion	Negative
<i>ROS1</i> fusion	<i>CD74-ROS1</i>
<i>RET</i> fusion	Negative
<i>NTRK1</i> fusion	Negative
<i>c-Met</i> amp	Negative
<i>c-Met 14</i> skipping mut	Negative

Abbreviations: FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; mut, mutation.

of postoperative adjuvant TKI therapy, according to the National Comprehensive Cancer Network clinical practice guidelines.

Limitations

Our study had some limitations. First, one case of a primary lung tumor included a dominance of adenocarcinoma cells with sarcomatoid differentiation, so we do not know whether or not both mutations were related to tumor tissue heterogeneity. Second, the response to TKIs in this patient is unknown because TKIs were not used.

Conclusion

We report a rare case of lung cancer in a patient harboring both an *EGFR* mutation and a *ROS1* fusion gene. The surgery and postoperative adjuvant chemotherapy showed a good response. For patients with this subtype, further research and experience are needed to summarize the biologic features and optimal modes of treatment, including targeted therapy in advanced lung cancer patients.

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Table 4 Clinical features of five patients with the *ROS1* fusion gene and *EGFR* mutation

Patient no	1	2	3	4	5 (present case)
Ethnicity	Chinese	Chinese	Chinese	Chinese	Chinese
Age (years old)	NA	NA	NA	NA	50
Sex	NA	NA	NA	NA	Female
Smoking history	Never smoker	Never smoker	Never smoker	Never smoker	Never smoker
Histology	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma with sarcomatoid differentiation
Primary lesion	NA	NA	NA	NA	The right inferior lung lobe
TNM staging	NA	NA	NA	NA	T _{2a} N ₀ M ₀ stage Ib
<i>EGFR</i> mutation status	I9del	I9del	I9del	20-ins	L858R
<i>ROS1</i> fusion partner	NA	NA	NA	NA	CD74
First-line treatment	Gefitinib	Gefitinib	NA	NA	Surgery
First-line treatment assessment	Partial response	Partial response	NA	NA	R ₀ resection ^a

Note: ^aComplete resection with no microscopic residual tumor.

Abbreviation: NA, not available.

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Disclosure

The authors report no conflicts of interest in this work.

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